IN THE UNITED STATES PATENT AND TRADEMARK OFFICE		
	Application Number	09/552,705
	Filing Date	April 19, 2000
	First Named Inventor	Shiuan CHEN et al.
	Group Art Unit	1652
	Examiner Name	C. Fronda
	Attorney Docket Number	2124-311
Title of the Invention:	*DRUG SCREENING USING A PROLINE-RICH NUCLEAR RECEPTOR CO-REGULATORY PROTEIN/NUCLEAR RECEPTOR CO-EXPRESSION SYSTEM*	

Declaration under 37 C.F.R. §132

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I, Shiuan Chen, do solemnly declare that:

1. I am the same Shiuan Chen named as an inventor on the above-referenced patent application.
2. I received a B.S. degree in chemistry in 1970 from the National Taiwan College of Marine Science and Technology, Keeling, Taiwan, Republic of China and a Ph.D. in biochemistry from the University of Hawaii, Honolulu, Hawaii in 1977. From 1978 to 1982, I began research at the University of Hawaii Department of Biochemistry and Biophysics. In 1982, I took the position of Research Assistant Professor at the School of Pharmacy at the University of Southern California, Los Angeles, California. Since 1985, I have been associated with the Beckman Research Institute of the City of Hope, Duarte, California, first as an Assistant Research Scientist, later as an Associate Research Scientist, and since 1994 as a Professor in the Division of Immunology. I have been studying various facets of nuclear receptor hormone function for approximately 15 years.
3. I have reviewed and am familiar with U.S. Patent Application Serial No. 09/552,705, filed April 19, 2000, Entitled "Drug Screening using a Proline-Rich Nuclear Receptor Co-Regulatory Protein/Nuclear Receptor Co-Expression

System," including the claims currently pending in the application. I also have reviewed and am familiar with the Office Action of January 10, 2002.

4. The assay methods of the present invention are designed to screen for compounds that affect nuclear hormone receptors such as the estrogen receptor. The co-regulatory protein (such as the proline-rich nuclear receptor co-regulatory protein (PNRC)) effectively is a reagent in the assay system since the PNRC is a component of the responsive functional unit which forms the basis of the assay technique. Nuclear receptors are known to play an important role in disease which is well established in the art and establishes a utility for the assay and for the products which are screened in the assay. This is and was well known and accepted in the art (see, for example, Jordan, Endocrin. Metab. 10(8):312-317, 1999; Bentram, Oncol. Res. 11(9):401-407, 1999; Levenson, Eur. J. Cancer 35(14):1974-1985, 1999). For example, through binding to estrogen, ER(estrogen receptor) plays a critical role in breast cancer development. Antagonists of ER (e.g., tamoxifen) are used as drugs to treat breast cancer. An embodiment of the assay method of the invention, in which the proline-rich nuclear receptor co-regulatory protein was used to screen the estrogen receptor, was able to identify Tamoxifen as a potent inhibitor of estradiol (E2) binding to the estrogen receptor (see Figure 1, attached hereto as Exhibit 2). This assay also has identified several new chemical compounds such as ICI 182780, which is exemplified in Figure 1 (attached hereto). These new compounds demonstrate potent Tamoxifen-like activity. Thus the assay has the utility of identifying compounds which can be used to treat breast cancer. In my opinion, this "real-world" utility would be accepted and recognized by the person of skill in the art.

5. This type of assay and the utility thereof is discussed in the specification at page 9, line 24 - page 10, line 11, providing a asserted utility as well as a well-established utility. The specification also discusses the utility of the claimed assay in the context of screening for proteins that interact with cancer drugs such as Tamoxifen, causing chemotherapeutic resistance in breast cancer. See specification at page 10, line 24 to page 11, line 4. Such a utility also is both

specific and well-established, and in my opinion provides a second asserted and well-established utility.

6. Many other nuclear receptors are known in the art and have been established to play a role in disease. Chemical entities which bind to and affect any of these known nuclear receptors may be identified by the assays which are disclosed in the present application. Thus, any nuclear receptor may be screened for protein/chemical pairs which modulate its activity. The screen is not merely, as the Office asserts, a method of finding proteins which can be used to research functions of nuclear receptors. The screen identifies compounds which can modulate the actions of the receptors that have well-established function in the body's physiology.

7. The Office states that the claims lack sufficient written description under 35 U.S.C. § 112, first paragraph because the specification does not sufficiently describe structural characteristics or properties of the chemical species to be screened. I have explained that the claimed method is an assay for screening the chemicals. Any chemical may be screened. Any library of chemicals may be screened. The structure of the chemical is not at all relevant to the method and the property of the chemicals is what is being screened, for example binding as known ligands bind or inhibiting binding of known ligands.

8. The "chemicals" listed by the Office at page 4 of the pending office action as representative of chemicals encompassed by the claims are chemicals which are already known to act as ligands. The Office therefore appears to conclude that the screening assay method is or should be limited to screening chemicals ready known to bind. The method is designed to identify chemicals which were previously not known to bind, not merely to confirm the receptor interactions of known ligands.

9. A person of skill in the art is familiar enough with screening assays to recognize that the value of a screening assay lies it its ability to screen any compound. Not all compounds yield a positive result in screens, but a screen is not unsupported by written description merely because all chemicals or classes of chemicals to be identified by the screen are not pre-identified and recited in the claims. Nor need the chemicals to be screened be defined by particular structural or functional

characteristics. It is the screening method itself which allows one to determine the important structural and functional characteristics of the ligand-candidates.

10. Any chemical may be a ligand candidate for a nuclear receptor, regardless of structure, known function or lack of known function. Indeed, many or most of chemicals which a person of skill might wish to screen by the claimed assay will have no known functional property at all, and could have any structure. The method itself will determine whether the chemical has the necessary structural property to bind to the nuclear receptors being assayed. Therefore it is not possible or desirable to pre-determine which chemicals or chemical classes will be screened for activity by the claimed method. If it were possible to know which chemicals productively interacted with nuclear receptors before screening, no screens would be necessary and all possible nuclear receptor ligands would already have been identified.

11. As with any screen, if only chemicals already known to interact with nuclear receptors were to be considered for screening, there would be no reason to screen the samples. The point of the screening is to determine whether or not there is an interaction with a particular chemical. Thus, a negative result in the screen does not mean that the screen does not work for its intended function. Any chemical can be screened because whether a particular chemical yields a positive or negative result does not affect whether the screen can be performed or is useful. The claims are not drawn to a method of assaying only samples which interact but to determine whether there is an interaction or not with a particular sample. Also, since it is never possible to pre-determine which sample will give a positive (or negative) result in any screening assay, the type of disclosure the Office seems to be requiring regarding chemicals to be screened simply can never be available, for any screening method. It therefore is my opinion that a requirement for this disclosure is not needed for a skilled person to recognize that we were in possession of the screening assay or to enable a skilled person to perform the screen.

12. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Shiuan Chen

May 10, 2002
Date

2124-311.de2

NAME	POSITION TITLE
Shiuan Chen	Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
National Taiwan College of Marine Science and Technology, Keelung Taiwan, Republic of China	B.S.	1970	Chemistry
University of Hawaii, Honolulu, Hawaii	Ph.D.	1977	Biochemistry

A. Positions & Honors:

1978 - 1981: Junior Researcher, Department of Biochemistry and Biophysics, University of Hawaii.

1981 - 1982: Assistant Researcher, Department of Biochemistry and Biophysics, University of Hawaii.

1982 - 1985: Research Assistant Professor, School of Pharmacy, University of Southern California, Los Angeles, CA.

1985 - 1988: Assistant Research Scientist, Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA.

1988 - 1994 Associate Research Scientist, Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA.

1994 - Present Professor, Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA.

National Service:

1. 1985 - Present Member of the City of Hope Cancer Center
2. 1996 - 2000 Member of the Reproductive Endocrinology Study Section, NIH
3. 2001 - 2005 Member of the Metabolic Pathology Study Section, NIH
4. 1996 - 1998, 2001 - Member of the Study Sections of the US Army Breast Cancer Research Program
5. 1998 - 2002 Member of the Research Committee, American Heart Association, Western States Affiliate
6. 2000 - 2001 Chairman of the Student Research Committee, American Heart Association, Western States Affiliate
7. 1998 - 1999 U.S. Environmental Protection Agency, Endoctrine Disruptor Study Section
8. 1999 - 2000 Member of the Study Section for Insight Award for Breast Cancer, NIH

B. Selected Peer Reviewed Publications

1. Mehta, L.K., Hobbs, S., Chen, S., Knox, R.J., and Parrick, J. Phthalimide analogs of CB 1954: synthesis and bioactivation. Anticancer Drugs 10:777-783, 1999.
2. Zhou, D., Quoch, K.M., Yang, C., Pohajdak, B., and Chen, S. PNRC: a proline-rich nuclear receptor co-regulatory protein that modulates transcriptional activation of multiple nuclear receptors including orphan receptors SF1 and ERRα1. Molecular Endocrinology, 14:986-996, 2000.
3. Zhou, D. and Chen, S. PNRC2 is a 16 kDa coactivator that interacts with nuclear receptors through a SH3 binding motif. Nucleic Acid Research, 29: 3939-3948, 2001.

4. Zhao, J., MaK, P., Tchoudakova, A., Callard, G., and Chen, S. Different Catalytic Properties and Inhibitor Responses of the Goldfish Brain and Ovary Aromatase Isozymes. General and Comparative Endocrinology, 123: 180-191, 2001.
5. Yang, C., Yu, B., Zhou, D., and Chen, S. Regulation of aromatase promoter activity in human breast tissue by nuclear receptors EAR-2, COUP-TF1 (EAR-3), and RARγ. J. Clin. Endo. Metab. Submitted, 2000.
6. Wu, K., Eng, E., Knox, R.J. and Chen, S. Direct demonstration of reductive activation and cytotoxic action of prodrug CB 1954 using DT-diaphorase transfected MDA-MB-231 breast cancer cell lines. Arch. Biochem. Biophys., 385:203-208, 2001.
7. Williams, D., Chen, S., and Young, M.K. Ratiometric analysis of the ferrocene boronate esters of 2- and 4-hydroxyestradiol by tandem electrospray mass spectrometry. Rapid Commun. Mass Spectrometry, 15:1-5, 2001.
8. Phornphutkul, C., Okubo, T., Wu, K., Harel, Z., Tracy, T. F., Pinar, H., Chen, S., Gruppuso, P. A., and Goodwin, G., Aromatase P450 expression in a female with isosexual precocious puberty due to feminizing adrenal adenoma. J. Clin. Endo. Metab., 86:649-652, 2001.
9. Okubo, T., Truong, T. K., Yu, B., Grube, B., Zhou, D., and Chen, S. Down-regulation of promoter I.3 activity of the human aromatase gene in breast tissue by zinc-finger protein, Snail (SnaH). Cancer Res., 61:1338-1346, 2001.
10. Okubo, T., Mok, S.C., and Chen, S. Regulation of aromatase expression in human ovarian surface epithelial cells. J. Clin. Endo.Metab., 85: 4889-4899, 2000.
11. Mor, G., Elisa, M., Song, J., Wiita, B., Chen, S., and Naftolin, F. Methyl-testosterone inhibits aromatase activity in JAR choriocarcinoma cells and macrophage-like THP-1 cells in culture. J. Steroid Biochem. Mole. Biol., in press, 2001.
12. Knox, R.J., Jenkins, T.C., Hobbs, S.M., Chen, S., Melton, R.G., and Burke, P.J. Bioactivation of 5-(Azirdin)-1-y1)-2,4-dinitrobenzamide (CB 1954) by Human NAD(P)H quinone oxidoreductase 2: A novel co-substrate-mediated antitumor prodrug therapy. Cancer Res., 60:4179-4186, 2000.
13. Kao, Y.-C., Korzekwa, K.R., Laughton, C.A., and Chen, S. Evaluation of the mechanism of aromatase cytochrome P450: A site-direct mutagenesis study. Eur. J. Biochem., 268:243-251, 2001.
14. Kao, Y.-C., Higashiyama, T., Sun, X., Yarborough, C., Choi, I., Osawa, Y., Simmen, F.A., and Chen, S. Functional characterization of porcino blastocyst and placental aromatase isoforms. Eur. J. Biochem., 267:6134-6139, 2000.
15. Grube, B.J., Eng, E.T., Kao, Y.-C., Kwon, A., and Chen, S. White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. J. Nutrition, 131: 3288-3293, 2001.
16. Faig, M., Bianchet, M.A., Talalay, P., Chen, S., Winski, S., Ross, D., and Amzel, L.M. Structures of recombinant human and mouse NAD(P)H:quinone oxidoreductases: species comparison and structural changes with substrate binding and release. Proc. Nat'l. Acad. Sci. USA, 97:3177-3182, 2000.
17. Eng, E.T., Williams, D., Mandava,U., Kirma, N., Tekmal, R.R., and Chen, S. Anti-aromatase chemicals in red wine. N.Y. Acad. Sci., in press, 2001.
18. Eng, E.T., Williams, D., Mandava,U., Kirma, N., Tekmal, R.R., and Chen, S. Suppression of aromatase (estrogen synthetase) by red wine phytochemicals. Breast Cancer Research and Treatment, 67: 133-146, 2001.
19. Chen, S., Zhou, D., Yang, C., Okubo, T., Kinoshita, Y., Yu, B., Kao, Y.-C., and Itoh, T. Modulation of aromatase expression in human breast tissue. J. Steroid Biochem. Mole. Biol., in press, 2001.
20. Chen, S., Zhou, D., Yang, C., and Sherman, M. Molecular basis for the constitutive activity of estrogen related receptor α-1 (ERRα-1). J. Biol. Chem., 276: 28465-28470, 2001.
21. Chen, S., Zhou, D., Kao, Y.-C., Yang, C., and Grube, B., Control of estrogen biosynthesis in breast cancer. Third Int'l Sym. on Hormonal Carcinogenesis., pp. 267-276, 2000.
22. Chen, S., Zhou, D., Kao, Y.-C., Okubo, T., Eng, E.T., Grube, B., Kwon, A., Yang, C., and Yu, B. Prevention and treatment of breast cancer by suppressing aromatase activity and expression. N.Y. Acad. Sci., in press, 2001.
23. Chen, S., Wu, K., and Knox, R. Structure-function studies of DT-diaphorase (NQO1) and NRH:quinone oxidoreductase (NQO2). Free Radical Biology and Medicine. 29:276-284, 2000.

24. Chen, S., DT-diaphorase. Encyclopedia of Molecular Medicine, in press, 2001.
25. Chen, S., CYP19 (Aromatase). Encyclopedia of Molecular Medicine, in press, 2001.

C. Research Support
Ongoing/Completed in the last 3 years

"Aromatase and Breast Cancer"
Principal Investigator: Shiuan Chen, Ph.D.
Agency: NIH, NCI
Type: RO1 (CA44735) Period: 2/1/88 - 12/31/04
The objective of this project is to generate information useful for controlling estrogen biosynthesis in breast cancer patient. A series of studies on the structure, expression and influence of aromatase in breast tissue are being performed. In nude mice, the investigators are studying growth, estrogen receptor status, and effects of aromatase inhibitors on tumors derived from cells overexpressing aromatase. Studies are being conducted to analyze the tissue specific regulation of promoter elements in the human aromatase gene. Yeast one-hybrid and two-hybrid analyses have been utilized to characterize transcription factors binding to the Cis-elements of the human aromatase gene. Ongoing studies on the structure of the active site of the aromatase enzyme will be continued, to generate information potentially useful for designing inhibitors or aromatase activity.

"Endocrine Disrupting Chemicals andAromatase"
Principal Investigator: Shiuan Chen, Ph.D.
Agency: NIH, NIEHS
Type: RO1 (ESO8258) Period: 9/1/96 - 12/31/03
Experiments are being carried out to provide a molecular and mechanistic basis as to howphytoestrogens and organochlorine compounds affect estrogen biosynthesis (i.e., aromatase function) in women. The structural requirement for the compounds to modify the catalytic properties of aromatase will be determined by computer modeling and by evaluation of their interactions with aromatase mutants. In addition, the effects of phytoestrogens and organochlorine compounds on aromatase expression throughout critical periods of exposure (neonatal, premenopausal, pregnant, and postmenopausal) are being investigated using cell culture studies with cell lines that utilize different promoters for aromatase expression.

"Breast Cancer Prevention withPhytoestrogens in Grape Juice"
Principal Investigator: Shiuan Chen, Ph.D.
Agency: University of California Breast Cancer Research Program
Type: Research Grant (4PB-0115) Period: 7/1/98 - 6/30/02

We plan to test the hypothesis that grape juice suppresses breast tumor growth by inhibitingaromatase. We will investigate the chemopreventive action of grape juice using two animal models: nude mouse and NMU-induction models.

"Evaluation of edible mushroomphytochemicals on aromatase activity and breast cancer cell proliferation."
Principal Investigator: Shiuan Chen, Ph.D.
Agency: American Institute of Cancer Research
Type: Research Grant, Period: 1/31/00 - 1/30/02

The aims of this project are: (1) To extract, identify and characterize the active component(s) in mushroom extract by standard biochemical and molecular techniques; (2) To characterize the inhibition kinetics of the components of mushroom extract on aromatase activity in an in vitro microsomal assay; (3) To evaluate mushroom extract on aromatase activity and cell proliferation as a function of dose, time reversibility, and dose schedule in an "in cell" model using an aromatase transfected breast cancer cell line; and (4) To evaluate the efficacy of mushroom extract on breast cancer tumor growth in vivo in tow vetebrate breast cancer tumor

models. These studies may identify a novel chemopreventive agent for breast cancer. They may lead to specific dietary recommendations incorporating edible mushrooms for chemoprevention of breast cancer.